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⑤④ **Optical assay: method and apparatus.**

⑤⑦ Optical assay apparatus (Figure 2) of the type comprising an optic waveguide (1; 1'; 1'') and a coating (7; 7') sensitized to a specific assay species. Light signal response is enhanced by coupling a resonant metallic medium (17; 17'; 17'') to the waveguide (1'; 1'') and to the sensitized coating (7; 7'). To this end a metal coated transparent buffer layer (17) may be interposed between the waveguide (1') and the sensitized coating (7). Alternatively (Figure 3), a metallised optically matched grating (17') can be used in place of the buffer layer (17); or, the metallic medium (Figure 4) can be of particulate form, each particle (17'') having a sensitized coating 7'.

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## OPTICAL ASSAY: METHOD AND APPARATUS

## TECHNICAL FIELD

The present invention concerns optical assay and relates in particular to a method and apparatus for detecting and/or monitoring or quantifying the presence and/or behaviour of specific assay molecular species in test fluid samples. The invention has application to, for example: immunoassay -the detection of antibodies, antigens, or hormones in blood samples; pollution monitoring; and, to the monitoring of clinical diagnostic reactions involving eg. enzymes and the like.

## BACKGROUND ART

Immunoassay techniques in the past have often required a complete schedule of analytical steps for species separation and identification. More recently, however, techniques have been developed to obviate the need for bulk separation. These latter rely on "in-situ" separation at the surface of a sensitized detector. Thus, for example, it is already known to detect the presence of antigens in blood samples by causing these antigens to be attracted into an adsorbing layer of a substance containing the appropriate antibody species, this layer lying adjacent to the gate of an insulated-gate field-effect transistor (IGFET) -a Chem-FET, so called. The current flow between the source and the drain of this transistor is modified in the presence of antigens in a test sample to which the gate layer is exposed. The transistor current is thus continuously monitored to detect the presence of antigens, and after test the transistor is disposed of.

There are however, limitations in the responsiveness of such Chem-FET devices, and the high cost of such disposable devices mitigates against widespread adoption.

More recently, sensitive, and possibly lower cost, optical assay techniques have been proposed. See for example: Proceedings of 2nd Optical Fibre Conference (Stuttgart 1984) page 75; "Detection of Antibody -Antigen Reactions at a Glass-Liquid Interface as a Novel Optical Immunoassay Concept", R.M.Sutherland, et al, of the Biomedical Group, Battelle, Geneva, Switzerland - (1984). In accordance with the technique that they describe, the complimentary antibody is covalently immobilized onto the surface of a planar or fibre optical waveguide. The reaction of immobilized antibody with antigen in sample solution is detected using the evanescent wave component of a light beam, totally internally reflected many times

within the waveguide. This evanescent wave has a characteristic penetration depth of a fraction of a wavelength into the aqueous phase, thus optically interacting with substances bound to or very close to the interface and only minimally with the bulk solution. The efficiency of this technique depends thus on tight confinement of the evanescent wave relative to the interface and this in turn requires a large differential in the value of refractive index each side of the interface.

## DISCLOSURE OF THE INVENTION

The present invention is intended as an alternative to that described above, is aimed at tight confinement of interactive power relative to the sensitized layer, but without necessary resort to a coupling medium of such high refractive index as aforesaid.

In accordance with this invention, therefore, there is provided an optical assay method comprising the following steps:

providing a coated metallic medium, the coating thereof being sensitized for a specific assay species;

generating in the metallic medium a resonant signal at optical frequency, the power of this signal extending into the sensitized coating;

providing coupling between said resonant signal and a monitored optical beam;

exposing the coated metallic medium to a fluid assay sample; and,

measuring any changes in the optical beam to detect a response to the presence of the specific assay species in the fluid sample.

In the aforesaid method tight confinement is achieved at the interface between the metallic medium and the coating. This medium may be, for example, a continuous metal film, and the resonant signal a surface plasmon wave. Alternatively, the metallic medium may be in the form of a dispersion of fine metal particles or a finely divided broken metal film, the resonant signal in this latter case resulting from Mie resonance. In both cases the resonant signal power is confined close to the surface of the metallic medium. It is therefore highly sensitive to the structure immediately adjacent to the surface and its sensitivity to, and penetration into, the sample bulk, effectively reduced.

In accordance with one aspect of this invention, therefore, there is provided an optical assay apparatus comprising:

a light source;

a light detector;

a propagating medium arranged relative to the source and detector for directing light from the source onto the detector,

a buffer medium of optically less dense material adjacent to a surface of the propagating medium;

a metallic medium, having the form of a continuous metal film, on the surface of the buffer medium remote from the propagating medium, and,

a coating, adjacent to the metallic medium, sensitized for a specific assay species.

In the above apparatus a plasmon surface wave is generated in the metallic medium by frustrated total internal reflection of the light beam incident from the source. As alternative to this method of wave coupling and generation, and in place of the buffer medium, the surface of the propagating medium may have the form of a grating of appropriate pitch with the metal film being formed directly thereon.

In accordance with an alternative aspect of this invention, there is provided an optical assay apparatus comprising:-

a light source;

a light detector;

a propagating medium, arranged relative to the source and the detector, for directing light from the source onto the detector; and,

a dispersion of fine metal particles, each contact with a material sensitized for a specific assay species.

In this latter apparatus the resonance in the fine metal particles (Mie resonance) involves a current pattern in each particle with a tightly confined associated external field.

The particles may be distributed in a solution - (eg. as a colloid) or may be confined to the surface of the propagating medium as a broken metal film. In each case optical excitation and detection is relatively straight forward, involving simply an appropriate incident optical beam.

## BRIEF INTRODUCTION OF THE DRAWINGS

In the accompanying drawings:

Figure 1 is a cross-section view of known optical assay apparatus,

Figures 2 to 4 are cross-section views of alternative optical assay apparatus each embodying the features of this invention; and,

Figure 5 is a schematic diagram to illustrate attenuation profiles for surface plasmon wave and evanescent optical wave for the apparatus of Figure 2 preceding.

## DESCRIPTION OF THE PREFERRED EMBODIMENTS

Embodiments of the invention will now be described, by way of example only, with reference to the drawings accompanying this specification.

In Figure 1 there is shown a known form of optical assay assembly in which light from a source S is directed into a planar waveguide 1 by means of a first coupling prism 3 and propagated by multiple total internal reflection to exit by means of a second coupling prism 5 where it is directed onto a light detector D. The external surface of the waveguide 1 is provided with a sensitized organic coating 7. This latter is exposed to a sample liquid 9 which is contained by means of a flow cell 11 and gasket 13 arrangement. In the coating 7, antibody material is covalently immobilised and this responds to any specific antigen material in the sample liquid to which it is exposed. The waveguide is of fused quartz material and this provides a large differential in optical density between the quartz waveguide 1 (high refractive index  $n_1$ ) and the adjacent coating 7 (low refractive index  $n_2$ ). Light is totally internally reflected within the body of the waveguide 1, a portion of the optical power, however, propagating as an evanescent wave in the coating medium 7. The binding of antigen by the immobilized antibody is monitored by a resultant increase in the light absorption measured at the detector D.

In the apparatus shown Figure 2, light of an appropriate frequency is directed from a source S onto a photodetector D via a propagating medium 1' - a prism (as shown) or an optical waveguide. The reflecting surface of this propagating medium 1' is covered by a thin spacer layer, a buffer medium 15 of optically less dense material and this latter carries a thin layer metallic medium, a continuous metal film 17. This metal film 17 is coated

with an organic layer 7, a layer sensitized for the specific assay species for which the apparatus is designed. The organic layer may comprise of, or may include, for example, an antigen for a specific antibody. In the course of use, the organic layer is exposed to a sample fluid 9. In operation, a surface plasmon is generated and sustained in the metallic medium 17. The power for this is supplied by frustrated total internal reflection of the light beam. The wave amplitude profiles for the light beam I, II and for the plasmon wave III are shown in Figure 5 as a function of displacement. In this figure, regions are delineated for the coating layer 7, the metallic medium 17, the buffer medium 15 and the propagating medium 1'. As shown, the light beam I corresponds to an evanescent wave II in the buffer medium 15. Coupling is provided between this latter and the plasmon wave III. The plasmon wave is tightly confined near to the surfaces of the metallic medium 17, as can be seen from the tail portions of the profile III of the plasmon wave. The thickness of the buffer medium is determined as an optimal solution. If too thick, the coupling is inordinately weak. If too thin, power losses are excessive. The metal film thickness is also chosen as optimal. If too thick the coupling is likewise too weak, and if too thin the plasmon energy is not confined as tightly as desired for this application.

Typical materials and layer thickness are given below:

Organic layer 7 ~ 100Å thick;

Metal film 17 100-400Å dependant on metal chosen;

Buffer layer 15 -low refractive index, low optical absorbtive material -eg. evaporated magnesium fluoride;

Propagating medium -of relatively high refractive index -eg. flint glass (n = 2).

Alternative coupling may be employed, using for example an optical grating. In the alternative apparatus shown in Figure 3 the reflecting surface of a prism 1" is formed as a grating 19 and a metal film 17' deposited directly onto the surface of this grating 19. The grating 19 may be defined in the material of the prism 1", or for convenience of construction, may be provided as a bonded component. The pitch  $\Lambda_1$  of the grating is chosen to provide a match between the plasmon wave of wavelength  $\Lambda_2$  and a light beam of appropriate wavelength  $\Lambda_3$ . The wavelength-pitch relationship for ideal matching is given as:-

$$\frac{1}{\Lambda_3} = \frac{1}{\Lambda_2} + \frac{1}{\Lambda_1}$$

Referring now to the apparatus shown in Figure 4, the metallic medium is provided as a colloid of fine metal particles 17" each of which has a sensitized organic coating 7". These particles are arranged so that they settle upon the reflecting surface of the propagating medium, prism 1'.

The presence of assay species may be detected and/or monitored by measuring changes in the absorption or polarisation of the monitored light beam. The interaction will depend on the frequency and angle of incidence of the light beam. Thus source S and detector D may be singular components mechanically scanned over a range of angles, or may each comprise an extended array each component being electrically addressed to simulate a scan. Alternatively, the source S and detector D may be set up in an optical static configuration. In certain applications, Raman scattering phenomena may be exploited, and assay species detected by monitoring frequency shift.

## Claims

1. An optical assay method comprising the following steps:-

providing a coated metallic medium, the coating thereof being sensitized for a specific assay species;

generating in the metallic medium a resonant signal at optical frequency, the power of this signal extending into the sensitized coating;

providing coupling between said resonant signal and a monitored optical beam;

exposing the coated metallic medium to a fluid assay sample; and,

measuring any changes in the optical beam to detect a response to the presence of the specific

assay species in the fluid sample.

2. An optical assay apparatus (Figure 2) comprising:

a light source (S);

a light detector (D);

a propagating medium (1') arranged relative to the source (S) and detector (D) for directing light from the source (S) onto the detector (D);

a buffer medium (15) of optically less dense material adjacent to a surface of the propagating medium (1'); and

a metallic medium (17), having the form of a continuous metal film, on the surface of the buffer medium (15) remote from the propagating medium (1'); and,

a coating (7), adjacent to the metallic medium (17), sensitized for a specific assay species.

3. An optical assay apparatus (Figure 3) comprising:-

a light source (S);

a light detector (D);

a propagating medium (1'') arranged relative to the source (S) and detector (D) for directing light from the source (S) onto the detector (D);

5 an optical grating (19) at the surface of the propagating medium (1'');

10 a metallic medium (17'), having the form of a continuous metal film, on the surface of the optical grating (19);

and,

15 a coating (7), adjacent to the metallic medium (17'), sensitized for a specific assay species.

4. An optical assay apparatus (Figure 4) comprising:-

20 a light source (S);

a light detector (D);

25 a propagating medium (1'), arranged relative to the source (S) and the detector (D), for directing light from the source (S) onto the detector (D); and,

30 a dispersion of fine metal particles (17''), each coated with a material (7') sensitized for a specific assay species.

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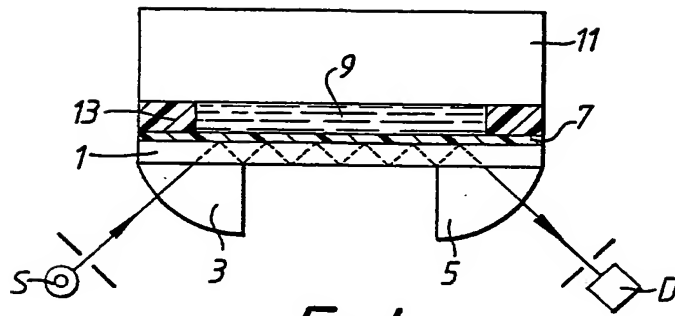
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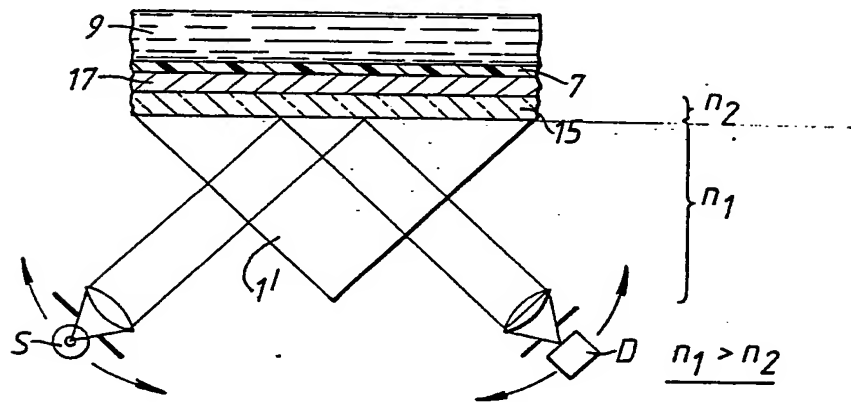
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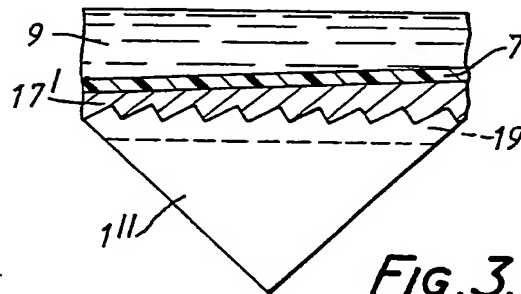
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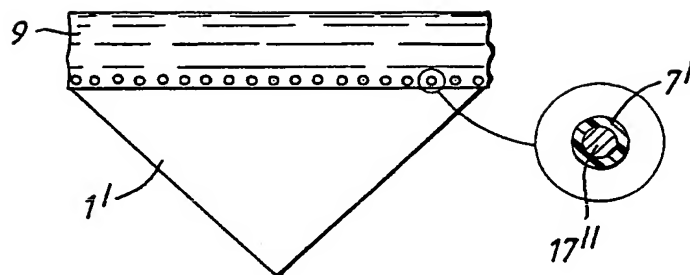
**FIG. 1.**  
PRIOR ART



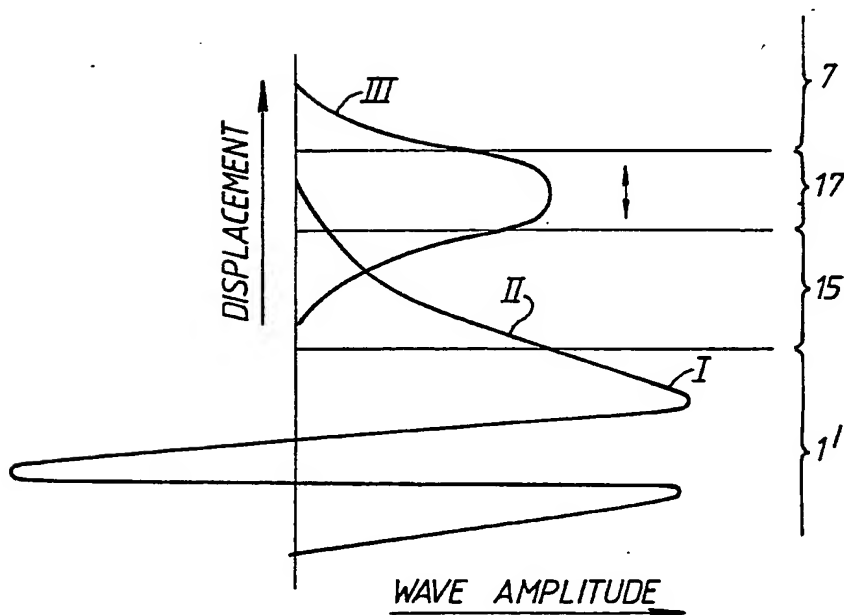
**FIG. 2.**



**FIG. 3.**



**FIG. 4.**



**FIG. 5.**

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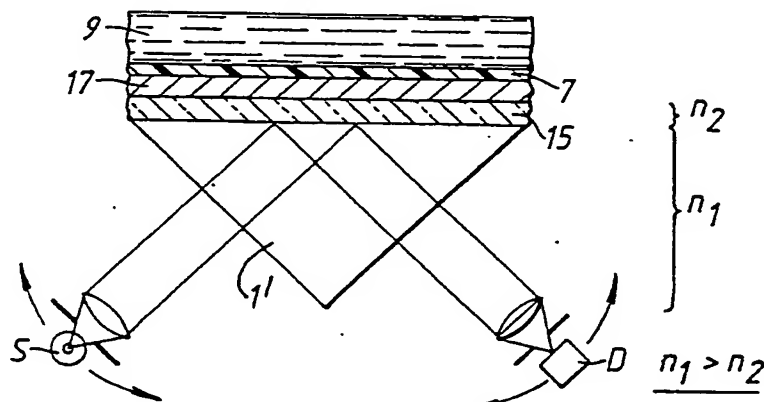


FIG.2.

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# EUROPEAN SEARCH REPORT

Application Number

EP 86 30 2651

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
X	SENSORS AND ACTUATORS, vol. 4, 1983, pages 299-304, Elsevier Sequoia, Amsterdam, NL; B. LIEDBERG et al.: "Surface plasmon resonance for gas detection and biosensing" * Whole document *	1	G 01 N 21/75 G 01 N 33/553
A	ANALYTICAL CHEMISTRY, vol. 54, no. 9, August 1982, pages 1071(A)-1080(A); I. CHABAY: "Optical waveguides" * Pages 1074A, 1077A; figure 4 *	1,2	
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A	IBM TECHNICAL DISCLOSURE BULLETIN, vol. 23, no. 11, April 1981, page 5099, New York, US; J. GORDON et al.: "Use of gratings to detect small quantities of materials by raman spectroscopy" * Whole document *	1,3	
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 21-02-1989	Examiner BOEHM CH.E.D.
<b>CATEGORY OF CITED DOCUMENTS</b> X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			



EP 86 30 2651

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A	ANALYTICAL CHEMISTRY, vol. 56, no. 9, August 1984, pages 1667-1668, Washington, US; T. VO-DINH et al.: "Surface-enhanced raman spectrometry for trace organic analysis" * Pages 1667-1668 * -----	1,4	
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 21-02-1989	Examiner BOEHM CH. E. D.
<b>CATEGORY OF CITED DOCUMENTS</b> X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

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